PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Frequency of exercise-induced ST-segment deviations and cardiac arrhythmias in recreational endurance athletes during a marathon race: results of the prospective observational Berlin Beat of Running study
AUTHORS	Herm, Juliane; Toepper, Agnieszka; Wutzler, Alexander; Kunze, Claudia; Kruell, Matthias; Brechtel, Lars; Lock, Juergen; Fiebach, Jochen; Heuschmann, Peter; Haverkamp, Wilhelm; Endres, Matthias; Jungehulsing, Gerhard; Haeusler, Karl Georg

VERSION 1 - REVIEW

REVIEWER	Martin Burtscher University of Innsbruck, Austria
REVIEW RETURNED	18-Jan-2017

GENERAL COMMENTS	Review of the manuscript entitled, “Exercise-induced ST-segment deviations and cardiac arrhythmias in leisure endurance athletes during a marathon race: Results of the Berlin Beat of Running study” This study (“Berlin Beat of Running study”) aimed at investigating the feasibility of ECG-monitoring and the occurrence of abnormal ECG findings during a marathon race. A total of 108 athletes have been studied and abnormal findings were demonstrated in 18 (17%) runners. Longer race times and advanced age were associated with abnormal ECG findings. Transient ST- deviations were related to elevated hsTnT values. The authors deal undoubtedly with an interesting issue from a scientific and clinical point of view. However, it is not really surprising or new that long-term Holter monitoring is feasible during marathon running which has for instance, recently been demonstrated by Grabs et al., Am Heart J 2015. Nevertheless, the relatively large sample and accompanied determinations of hsTnT values and MRI make the study important but several concerns have to be addressed before a final recommendation can be made: 1. In my opinion, there is no clear hypothesis stated. What did the authors expect to find? The feasibility seems rather clear than the types and frequency of abnormal ECG findings. 2. How can you exclude a severe selection bias due to your recruitment procedure? 3. Do you have information on current medications? 4. Although there is an association between ST segment deviations and hsTNT values how can you really differentiate between true silent ischemia and false positive results due to artefacts? 5. 4 runners reported palpitations; which type of arrhythmias were responsible for that?

	6. Please, report values of biochemical variables pre, post1 and post2 (to see whether some of them returned to baseline). 7. It would also be interesting to perform multivariate logistic regression analysis with abnormal ECG findings as independent variable. 8. Please, present tables showing results of univariate and multivariate logistic regression analyses including all studied variables. 9. It should be more highlighted that you studied a pretty fit and active population running about 60 km per week and used to marathon running. Would you expect more abnormal ECG findings in a less active population?

REVIEWER	Antonis S. Manolis, MD Athens University School of Medicine, Athens, Greece
REVIEW RETURNED	21-Jan-2017

GENERAL COMMENTS	In the present study, Herm J et al studied master athletes, 108 marathon runners, aged 45-53 years, by performing blood studies before and after a marathon race and ECG monitoring during the race with a portable Holter device. They found ECG abnormalities during the marathon in 18 (16.8%) athletes, >/=1 episode of non-sustained VT (NSVT) in 10 (9.3%), atrial fibrillation (AF) in 1 and transient ST-segment deviations in 8 (7.5%) athletes. High-sensitivity cardiac troponin T (hs-cTnT) was elevated in 18 (16.7%) athletes, associated with ST-segment depression (odds ratio - OR 11) and a longer marathon finishing time (OR 1.5 per 30 min). This is an important study confirming prior studies (see ref No 9, 11, 12, 14, 23, 24) that have reported similar consequences of extreme exercise that may be potentially harmful, albeit there were no clinical correlations with these findings, hence of dubious clinical importance. However, this was probably the result of meticulous pre-participation screening for underlying cardiovascular diseases that these patients might have had prior to enrollment, and this needs to be clarified by the authors. The authors demonstrated that ECG monitoring is feasible in athletic activities in a larger cohort than previous studies (ref No 12, 15, 30), however we also need to know of the opinion of the athletes, whether it was intrusive, slowing them down, or uncomfortable to wear this monitor, and also a comment on the specific logistics of fitting the device, why the arm vs the waist, etc.; the technical problems involved with such motion and perspiration creating artifacts and interpretation difficulties. How does this compare with other technologies, e.g. wireless? Specifics are also needed regarding the NSVT, mean number of beats, rate, duration; duration of AF. High-sensitivity cardiac troponin (hscTn) values are now reported in ng/L instead of μg/L and for the troponin T the normal range has been considered to be up to 14 ng/L, borderline values 14-53 ng/L and abnormal values >53 ng/L (Am Heart J 2010;159:933–936). The authors considered a cut-off of 50 ng/L; was this based on a reference range obtained in their laboratory (?), suggested by the manufacturer(?); what was the mean and range of cTnT values in the 18 patients having an abnormal value? Did this correlate with a rise in the CK values as well, in this group? Were there any heat exhaustion signs in these

	individuals? How high were these values and were they related to dehydration and increased creatinine levels? cTnT levels were apparently not related to training experience, as some other studies have indicated (ref 24). The authors did a cardiac MRI study in a very small subset, but unfortunately this was done too late after the end of the race to detect any transient or reversible abnormalities; nevertheless, these studies performed at 10-42 days later, were negative. It would have been interesting to see echocardiographic findings, much easier to perform, immediately upon finishing the race in the athletes with the abnormal findings. Furthermore, no mention is made about further pursuing assessment of possible ischemic findings in these patients with performance of myocardial scintigraphy and/or CT or regular coronary angiography in these individuals to more definitively exclude coronary artery disease, at least for those with transient ST depression. Were the 9 hypertensive individuals included in this study, which rather should have been excluded from the study, the ones who had the transient ST changes? Did the authors perform regular exercise testing after the race in the individuals with abnormal findings to see whether they were reproducible? Some other assessment examinations that might also have been important in this cohort may include thyroid function studies, and also Holter monitoring and exercise testing before the race that could have provided clues for catecholamine-sensitive arrhythmias, inducible ischemia or hypertensive response, etc. Finally, it would have nice to have follow-up data in these patients to see whether any arrhythmic or cardiovascular events ensued during further long-term follow-up, particularly in those with abnormal ECG findings during the race. The above notwithstanding, the importance of the present study relates to the fact of being able to monitor athletes real-time during their athletic activities, which may enhance our understanding of the effects of strenuous exercise on the cardiovascular system. While exercise can promote health when moderate and regular and performed with gradually increased intensity, it can produce harm when too strenuous and of prolonged duration, specifically incurring cardiac injury and/or dysfunction and consequent electrical instability (Pacing Clin Electrophysiol 2016;39:748-62).

REVIEWER	Eduard Guasch IDIBAPS, Spain.
REVIEW RETURNED	28-Jan-2017

GENERAL COMMENTS	In this manuscript, Herm et al. report the findings of the “Berlin Beat of Running” study. A cohort of >100 well-trained marathon runners were ECG-monitored and had TnT measured during the Berlin marathon in 2011. They find that one in six athletes develop ECG abnormalities during the race (most commonly nsVT in 9% and ST-segment deviations in 7% of athletes). Interestingly, those with increased TnT after the race were 11-fold more prone to have had ST-segment changes. A cardiac magnetic resonance was performed in 10 athletes (obtained >10 days after the race), in whom no abnormalities were found. The results are interesting.

	The authors should provide a clearer definition of their predefined aims in the introduction. As of now, this is only vaguely addressed. ECG filtering plays a critical role in ST-segment interpretation and may lead to a large number of false positives (Tayler D et al. Br Heart J 1985;54:121-128; Abächerli R, Schmid HJ J Electrocardiol. 2009;42:574-9; Buendia-Fuentes et al. ISRN Cardiology 2012:706217). The authors state that the filtering parameters of the monitors allowed to evaluate ST-T wave changes (pg 8, line 15). The filter settings and the specifications should be reported. Also, the authors should report which leads they recorded. Athletes with ST-segment depression at any time during the race are considered abnormal, but this may encompass a large variability (from very short time in some athletes to much longer time-periods in others). Providing results on the duration and the intensity of ST segment depression would substantially improve the manuscript. Moreover, the athletes carried the Holter monitor for >100 hours before and after the marathon. Were ST-segment changes found at any time outside the marathon period? An important issue is the significance of ST-segment deviation during a marathon; unfortunately tests aiming at ruling out an ischemic heart disease were not carried out. However, I feel that the specificity and sensitivity for ischemic heart disease of ST-segment deviation in Holter recordings should be more thoroughly discussed to address its significance in athletes. The mechanisms behind Tn elevation after a marathon race have been widely studied in the literature, but up-to-date evidence points to increased permeability of myocytes as a central event (Eijsvogels et al. Physiological Reviews 2016;96:99-125). The authors found a high correlation between ST-segment changes and TnT elevation; do they think that ST-segment changes and Tn elevation are both caused by the same factors? Could athletes with both ST-segment deviation and high Tn be at a higher risk of ischemic heart disease? In table 2, and throughout the manuscript, the authors classify athletes with and without an “abnormal ECG”. This concept mixes ST-segment depression and nsVT. In my opinion this classification may be misleading as the origin and mechanisms of both events is likely completely different. The authors speculate that transient troponin elevation, ST-segment alterations and nsVT are benign (pg 11 line 30) on the grounds of their results. However, the authors would need to provide follow-up data showing similar outcomes in those with and without such events to prove benignity. It should be emphasized that a normal cardiac MRI >10 days after does not completely rules out exercise-induced damage. While fibrosis is likely diffuse in athletes (Benito et al. Circulation 2011;123:13-22), this was not addressed with specific cardiac MR techniques i nthe Herm et al. manuscript. Further, most changes occurring just during a marathon race have regressed 6 to 11 days after the race (La Gerche et al. Eur Heart J 2012;33:998-1006). Minor comments:

	Although I assume that a 1 mm was used as a threshold for ST-segment depression, this should be specifically reported in the manuscript. Herm et al. state that incidence of myocardial infarction after running a marathon is limited to case reports" (pg4 , line 23), but this was addressed in a recent French registry (Gerardin et al. European Heart Journal 2016;37:2531–2541).

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

This study ("Berlin Beat of Running study") aimed at investigating the feasibility of ECG-monitoring and the occurrence of abnormal ECG findings during a marathon race. A total of 108 athletes have been studied and abnormal findings were demonstrated in 18 (17%) runners. Longer race times and advanced age were associated with abnormal ECG findings. Transient ST- deviations were related to elevated hsTnT values.

The authors deal undoubtedly with an interesting issue from a scientific and clinical point of view. However, it is not really surprising or new that long-term Holter monitoring is feasible during marathon running which has for instance, recently been demonstrated by Grabs et al., Am Heart J 2015. Nevertheless, the relatively large sample and accompanied determinations of hsTnT values and MRI make the study important but several concerns have to be addressed before a final recommendation can be made:

1. In my opinion, there is no clear hypothesis stated. What did the authors expect to find? The feasibility seems rather clear than the types and frequency of abnormal ECG findings.

We thank the reviewer for this comment. We have added the following statement to the Methods section (on page 4-5):

"[…] The study protocol is in accordance with the Helsinki declaration and was approved by the Ethics Committee of the Charité - Universitätsmedizin Berlin (EA4/042/11). The primary hypothesis was: Cardiac arrhythmias and especially AF is frequently found in experienced marathon runners. Therefore, the primary outcome is the number of marathon runners with newly diagnosed cardiac arrhythmias. The main secondary hypotheses were: (1) There are predictable risk factors associated with cardiac arrhythmias in marathon runners; (2) Pathological laboratory findings are (in part) associated with cardiac arrhythmias;

(3) Marathon runners with elevated troponin levels do not have MRI-detected myocardial scars suggestive for myocardial infarction. […]"

2. How can you exclude a severe selection bias due to your recruitment procedure?

This is an important issue raised by the reviewer. The recruitment process was described in short in the manuscript ("All pre-registered participants were informed by the organizers of the marathon about the study, and they contacted the study personnel if they were interested. 110 Participants aged 35-60 years with at least 2 marathons runs within the last 5 years and an average training of 40 km running per week were enrolled after giving written informed consent."). While athletes with a particular interest in health issues may have preferably volunteered, we cannot exclude a potential selection bias.

We have added this limitation to the Discussion section (on page 13):

"[…] Since only ~~25%~~one fourth of all athletes were female and because of a potential selection bias during enrolment the generalizability of our results is limited. […]"

3. Do you have information on current medications?

We assessed the current medication at enrolment and have now added the respective information in Table 1 (on page 26).

Table 1: Baseline characteristics of the 107 participants of the "Berlin Beat of Running" study who finished the marathon and who had evaluable ECG data.

Age; mean; years; median [IQR]	48 [45-53]
Female gender; % (n)	24.3 (26)
[…]	
Medication at enrolment	

Antiplatelet; % (n)	0.9 (1)
Oral anticoagulant; % (n)	0
Beta-blocker; % (n)	1.9 (2)
Statin; % (n)	1.9 (2)
Antihypertensive; % (n)	6.5 (7)

4. Although there is an association between ST segment deviations and hsTNT values how can you really differentiate between true silent ischemia and false positive results due to artefacts?

We thank the reviewer for this comment. We have validated ST-segment deviations in virtually motion- and perspiration-artefact free ECG recordings in all athletes. In particular, ST-segments were analyzed in relation to the TP segment. Segments were analyzed after recording of stable isoelectric TP segments in three consecutive beats. If single QRS complexes showed notching, slurring or fragmentation, QRS and ST intervals were excluded from further analysis. In our opinion, these precautions minimize an "artefact bias" which, however, cannot be excluded.

We extensively discussed the concern of the reviewer. Finally, we came out with the conclusion that we cannot be sure that the observed ST-segment deviations are definitively based on silent ischemia. This issue is intensively addressed in the revised manuscript.

We have added the following statement to the Methods section (on page 5):

"[...] The ST-segment was considered abnormal in the virtually artifact free 2-lead ECG if horizontal or down-sloping ≥ 1mm occurred over the 60 ms after the J-junction (80 ms if the heart rate was <120 beats/min) [18]. ST-segments were analyzed in relation to the TP segment. Segments were analyzed after recording of stable isoelectric TP segments in three consecutive beats. If single QRS complexes showed notching, slurring or fragmentation, QRS and ST intervals were excluded from further analysis."

In addition, the following information was added to the Discussion section (on page 11 & 12):

"Moreover, this is the first study reporting an association of transient ST-segment abnormalities during a marathon with elevated hsTNT levels after finishing the race. However, we cannot be sure that the observed ST-segment deviations are definitively based on silent ischemia."

[…]

Our results strengthen the assumption that hsTnT elevation originates from the heart and not primarily from non-cardiac sources [8]. However, we are unable to draw final conclusions."

5. 4 runners reported palpitations; which type of arrhythmias were responsible for that?

Of the four runners reporting palpitations during the marathon race a single athlete had documented atrial fibrillation, one athlete had non-sustained ventricular tachycardia (lasting nine beats) and one athlete had multiple supraventricular premature beats (62/hour). In one athlete reporting palpitations, we did not observe any ECG abnormalities.

We have now included this information in the Results section (page 8):

"[…] While four (3.7%) athletes reported palpitations during the marathon race, one of them had AF, one had a single nsVT (lasting nine beats) and another one had multiple supraventricular premature beats (62/hour)."

6. Please, report values of biochemical variables pre, post1 and post2 (to see whether some of them returned to baseline).

To better address this issue, we have added a table (to be published in the *online supplement*) depicting the baseline characteristics and values of biochemical variables of all 21 athletes with either ST-segment deviation and/or hsTnT elevation.

While high-sensitive troponin T values returned to normal within up to 58 hours after the race in almost all athletes (95%), creatine kinase remains elevated in all 21 athletes (with ST-segment deviation and/or hsTnT elevation).

The following information was added to the Results section (on page 8 and 9):

"Characteristics of all athletes with ST-segment deviation are displayed in the *online supplement*. […] Characteristics of all athletes with elevated hsTnT deviation are displayed in the *online supplement*."

ONLINE SUPPLEMENT

High-sensitive troponin T (hsTnT) values returned to normal within up to 58 hours after the marathon race in almost all athletes (95%). Creatine kinase (CK) remains elevated in all 21 athletes (with ST-segment deviation and/or hsTnT elevation).

Table ONLINE SUPPLEMENT: Characteristics of 21 athletes with either ST-segment deviation (>1 mm) and hsTnT elevation (≥50 ng/L) (n=5), ST-segment deviation (n=3) or hsTnT elevation (n=13). Pathological findings are labeled in bold.

Age	Sex	Medication	Cardiovascular risk factors	NSVT[§]	ST-segment deviation	hsTnT[$] ng/L[*]	hsTnT ng/L[**]	CK-MB mg/dL[*]	CK-MB mg/dL[**]	CK mg/dL[*]	CK mg/dL[**]	Cardiac MRI
45	Female	None	No	No	**Yes**	**91**	19	21	32	877	1194	Yes
45	Female	L-Thyroxin	No	No	**Yes**	**71**	12	18	16	243	235	Yes
53	Male	None	No	No	**Yes**	**63**	14	14	35	893	2121	Yes
59	Male	None	No	No	**Yes**	**66**	12	19	54	406	2236	Yes
60	Male	None	Smoker	**Yes**	**Yes**	**70**	12	27	72	2867	3625	Yes
48	Male	None	No	No	**Yes**	32	16	18	44	381	1719	No
55	Male	None	No	No	**Yes**	21	12	21	35	201	489	No
60	Male	Antihypertensive	Hypertension	No	**Yes**	27	13	28	44	767	1979	No
44	Female	None	Smoker	No	No	**78**	12	26	20	306	192	Yes
48	Male	None	No	No	No	**138**	20	59	67	325	2186	Yes
49	Female	None	No	No	No	**63**	12	22	41	205	1357	Yes
50	Male	None	No	No	No	**87**	29	30	73	360	3462	Yes
60	Female	None	No	No	No	**216**	64	33	14	455	169	Yes
39	Male	None	No	No	No	**59**	23	47	87	583	2756	No
40	Female	None	No	No	No	**85**	12	42	33	696	914	No
45	Male	None	No	No	No	**83**	29	175	157	195	605	No
47	Male	None	No	No	No	**52**	12	37	42	309	1983	No
48	Male	None	No	No	No	**64**	12	52	101	405	4604	No
50	Female	Antihypertensive	Hypertension	No	No	**50**	12	29	28	315	485	No
53	Female	None	No	No	No	**62**	12	30	24	339	448	No
54	Male	None	No	No	No	**67**	15	33	23	234	409	No

[§] non-sustained ventricular tachycardia; [$] high-sensitive Troponin T; [*] within 30 minutes post-race; [**] up to 58 hours after the race

7. It would also be interesting to perform multivariate logistic regression analysis with abnormal ECG findings as independent variable. Please, present tables showing results of univariate and multivariate logistic regression analyses including all studied variables.

We thank the reviewer for his/her comment. We have now performed a multivariate analysis as requested. Analyzing potential impact factors on abnormal ECG findings, we identified age and marathon time being statistically significant on a p=0.05 level in univariate analysis. In multivariate analysis, advanced age remained significant (OR 1.11 per year [95%CI 1.01-1.23]).

Analyzing potential factors influencing hsTnT elevation, we identified ST-segment deviation, marathon time and hematocrit (measured immediately after the race) being statistically significant according to univariate analysis. In multivariate analysis, ST-segment deviation remained statistically significant (OR 9.9 [95%CI 1.9-51.5]) as well as hematocrit (OR 0.76 per percent [95%CI 0.62-0.92]).

According to the advice of the reviewer we included these results in the revised manuscript (Abstract, Methods section, Results section & Table 2 & 3). In addition, we have revised the statement in the limitation section (on page 13):

"Moreover, due to the limited number of endpoints observed, we believe that the results of the multivariate analysis should be interpreted with caution. ~~a valid multivariate analysis was not possible due to the limited number of endpoints observed, which was due to the complex nature of the study~~"

Abstract (on page 2):

Abnormal ECG-findings were associated with ~~finishing time (OR 1.70 per 30 minutes [95%CI 1.18-2.43]) and~~ advanced age (OR 1.11~~5~~ per year [95%CI 1.01~~5~~-1.23~~7~~]); sex and cardiovascular risk profile had no impact. Directly after the race, high-sensitive troponin T was elevated in 18 (16.7%) athletes and associated with ST-segment deviation (OR 9.9 [95%CI 1.9-51.5])~~11.0 [95%CI 2.35-51.7])~~ ~~and a longer marathon finishing time (OR 1.5 per 30 minutes [95%CI 1.03-2.07])~~, while age, sex and cardiovascular risk profile had no impact.

Methods section (on page 7):

"In multivariate analysis, potential impact factors identified on a $p < 0.05$ level in univariate analysis were entered in a binary logistic regression model using backwards selection."

Results section (on page 9 and page 10):

In univariate analysis, advanced age (~~OR 1.15 per year [95%CI 1.05-1.27]; p=0.004~~) and a longer marathon finishing time (~~OR 1.70 per 30 minutes [95%CI 1.18-2.43]; p=0.009~~) were associated with abnormal ECG findings, while sex, cardiovascular risk profile, hematocrit post-race and the number of previous marathons were not. In multivariate analysis, advanced age remained significant (OR 1.11 per year [95%CI 1.01-1.23]) (Table 2).

[…]

In runners with elevated hsTnT, we found more frequent ST-segment deviations (~~OR 11.0 [95%CI 2.35-51.7]; p<0.0001~~) but no association with cardiac arrhythmias. In addition, elevated hsTnT was found in individuals with a longer marathon finishing time (~~OR 1.5 per 30 minutes [95%CI 1.03-2.07]; p=0.040~~). Athletes with elevated hsTnT had a lower hematocrit compared to athletes without hsTnT elevation (p=0.003). In multivariate analysis, ST-segment deviation (OR 9.9 [95%CI 1.9-51.5]) as well as hematocrit (OR 0.76 per percent [95% CI 0.62 – 0.92]) remained statistically significant.

Discussion section (on page 11 and 12):

"Advanced age ~~or longer marathon finishing time~~ was associated with abnormal ECG findings (**Table 2**)."

[…]

Interestingly, elevation of hsTnT was not related to age, sex, training status, the cardiovascular risk profile or the presence of cardiac arrhythmias during the race, but was related to exercise-induced ST-segment deviation ~~(p<0.0001) and longer marathon finishing time (p=0.040)~~ and inversely correlated with the hematocrit (measured immediately after the race)."

Conclusion (on page 14):

"Cardiac arrhythmias or exercise-induced ST-segment deviations appear in a relevant subset of experienced leisure athletes during a marathon race and, predominantly in older ~~and~~ athletes ~~who are less fit~~.

Table 2: Cardiovascular risk profile and training status in leisure athletes with or without abnormal ECG findings, respectively. ST-segment deviation, atrio- or ventricular arrhythmias (atrio-ventricular block grade IIb or III, triplets, non-sustained ventricular tachycardia, supraventricular tachycardia) or atrial fibrillation were regarded as abnormal findings.

	Normal	**Abnormal ECG**	**Univariate Analysis**	**Multivariate Analysis**

	ECG			
	n=89	n=18	**p-value ***	**OR (95%CI)**
Age; years; median [IQR]	48 [44-50]	54 [48-59]	0.004	1.11 (1.01-1.23)
[…]				
Present marathon time; min; median [IQR]	238 [215-268]	275 [229-326]	0.009	1.44 (0.98-2.12)

*Values are expressed in % (n), mean ± SD or median [IQR] as appropriate; * p-value calculated by chi^2-test or Mann-Whitney U test, as appropriate. Multivariate analysis was calculated in a binary logistic regression model using backwards selection.*

Table 3: Troponin T elevation post-marathon in the 107 athletes who finished the marathon race and who had evaluable ECG data.

	Troponin T	**Troponin T**	**Univariate Analysis**	**Multivariate Analysis**
	< 50 ng/L	**≥ 50 ng/L**	**p-value ***	**OR (95%CI)**
	n=89	n=18		
[…]				
Present marathon time; min; median [IQR]	236 [217-269]	268 [237-309]	0.040	1.25 (0.83-1.87)
[…]				
ST-segment deviation; % (n)	3.4 (3)	27.8 (5)	<0.0001	9.9 (1.90-51.5)
Hematocrit post-race; %; median	44 [42-46]	41	0.003	0.76 (0.62-0.92)

[IQR]	[39-44]

*Values are expressed in % (n), mean ± SD or median [IQR] as appropriate; * p-value calculated by chi² – test or Mann-Whitney U test, as appropriate. Multivariate analysis was calculated in a binary logistic regression model using backwards selection.*

8. It should be more highlighted that you studied a pretty fit and active population running about 60 km per week and used to marathon running. Would you expect more abnormal ECG findings in a less active population?

This is an interesting issue raised by the reviewer. As old age and longer marathon finishing time were associated with abnormal ECG findings in our study, one might speculate that there might have been more abnormal ECG findings in a less active population. However, further studies are needed to validate this assumption. As stated in the Discussion section (on page 13), the generalizability of our results is limited.

We revised the manuscript in the Discussion section (on page 12) to better characterize the study cohort:

"[…] Taken together, vigorous exercise can go along with transient troponin elevation, ST-segment alterations or nsVT in a fit and active population. […]"

Reviewer 2:

In the present study, Herm J et al studied master athletes, 108 marathon runners, aged 45-53 years, by performing blood studies before and after a marathon race and ECG monitoring during the race with a portable Holter device. They found ECG abnormalities during the marathon in 18 (16.8%) athletes, >/=1 episode of non-sustained VT (NSVT) in 10 (9.3%), atrial fibrillation (AF) in 1 and transient ST-segment deviations in 8 (7.5%) athletes. High-sensitivity cardiac troponin T (hs-cTnT) was elevated in 18 (16.7%) athletes, associated with ST-segment depression (odds ratio - OR 11) and a longer marathon finishing time (OR 1.5 per 30 min).

1. *This is an important study confirming prior studies (see ref No 9, 11, 12, 14, 23, 24) that have reported similar consequences of extreme exercise that may be potentially harmful, albeit there were no clinical correlations with these findings, hence of dubious clinical importance. However, this was probably the result of meticulous pre-participation screening for underlying cardiovascular diseases that these patients might have had prior to enrollment, and this needs to be clarified by the authors.*

   This is an important issue raised by the reviewer. The extent of pre-participation screening for underlying cardiovascular diseases was not assessed in detail after enrolment. Therefore, we cannot exclude a potential bias and acknowledge this issue in the limitations of the Discussion section (on page 13).

   "Since only ~~25%~~ one fourth of all athletes were female and because of a potential selection bias during enrolment the generalizability of our results is limited."

2. *The authors demonstrated that ECG monitoring is feasible in athletic activities in a larger cohort than previous studies (ref No 12, 15, 30), however we also need to know of the opinion of the athletes, whether it was intrusive, slowing them down, or uncomfortable to wear this monitor, and also a comment on the specific logistics of fitting the device, why the arm vs the waist, etc.; the technical problems involved with such motion and perspiration creating artifacts and interpretation difficulties. How does this compare with other technologies, e.g. wireless?*

   No athlete reported discomfort by wearing the ECG device during the race and no athlete chose to stop wearing the ECG device prematurely. Wearing the device on the upper arm was preferred by most athletes, as many were used to wear digital entertainment systems or a smartphone during

exercise in a similar fashion. As stated in the Results section (on page 7) “data quality was sufficient was sufficient to ensure assessment of arrhythmias and ST-segment deviations in 107 (98.2%) athletes” despite of motion and perspiration artifacts were frequently found. Unfortunately, we have no experience with wireless technologies.

We have included the following information in the Results section (on page 7) of the revised manuscript:

“[…] Data quality of long-term Holter-ECG was sufficient to ensure assessment of arrhythmias and ST-segment deviations in 107 (98.2%) athletes, although motion and perspiration artifacts were present in the majority of athletes. In one athlete, a technical error occurred. Consistently wearing the ECG recorder on the upper arm using a carrier bag, ~~the other~~ no athlete~~s~~ reported ~~no~~ problems in this regard. No athlete stopped wearing the ECG device prematurely. […]”

3. *Specifics are also needed regarding the NSVT, mean number of beats, rate, duration; duration of AF.*

We thank reviewer for pointing out that we have omitted this information. We have now included the respective information in the Results section (page 8):

“[…] We observed nsVT in 10 (9.4%) athletes (Figure 1), 2 (20%) of those athletes were female. In athletes with nsVTs the median number of beats was 3 (IQR 3-5; range 3-9), median rate was 166 beats per minute (IQR 149-188; range 133-224) and median duration of the recorded nsVT was 1121 ms (IQR 919-1841; range 901-4400). We did not observe an AV block or a SVT. Persistent atrial fibrillation was found in one male patient (0.9%).”

4. *High-sensitivity cardiac troponin (hsTnT) values are now reported in ng/L instead of µg/L and for the troponin T the normal range has been considered to be up to 14 ng/L, borderline values 14-53 ng/L and abnormal values >53 ng/L (Am Heart J 2010;159:933–936). The authors considered a cut-off of 50 ng/L; was this based on a reference range obtained in their laboratory (?), suggested by the manufacturer(?); what was the mean and range of hsTnT values in the 18 patients having an abnormal value? Did this correlate with a rise in the CK values as well, in this group? Were there any heat exhaustion signs in these individuals? How high were these values and were they related to dehydration and increased creatinine levels? hsTnT levels were apparently not related to training experience, as some other studies have indicated (ref 24).*

We thank reviewer for pointing out that ng/L should be used instead of µg/L and have changed this unit of measurement throughout the revised manuscript. The cut-off 50ng/L was chosen because of the given standard at the Charité, Berlin, Germany.

Two athletes presented with hsTnT levels of 50-53 ng/L. Both had no ST-segment deviation or nsVT as depicted in the additional table (online supplement).

As depicted in Table 3, creatinine levels, creatine kinase (CK) levels or CK-MB levels after the marathon race did not differ in athletes with or without hsTnT levels of ≥50 ng/L or <50 ng/L, respectively. The median hsTnT value in 18 athletes with hsTnT ≥50 ng/L immediately after the race was 68.5 ng/L (IQR 62.8-85.5, range 50-216). As "temperatures reached a maximum of 22 degrees Celsius" during the marathon, heat exhaustion was obviously not a major issue. While hsTnT levels did not correlate with weekly training status, we observed an *inverse* correlation of elevated hsTnT levels and the hematocrit [median 40.7% (IQR 38.9-44.8) in athletes with hsTnT elevation vs. median 43.8% (IQR 42.3-45.7) in athletes without hsTnT elevation, p=0.003)].

We have added the following information to the Results section and the Discussion section and in Table 3:

Results section (on page 9):

"[…] Median hsTnT values in 18 athletes with elevated hsTnT was 68.5 ng/L (IQR 62.8-85.5, range 50-216)."

[…]

"Athletes with elevated hsTnT had a lower hematocrit (median 40.7% (IQR 38.9-44.8)) compared to athletes without hsTnT elevation (median 43.8% (IQR 42.3-45.7); OR 0.75 per percent [95%CI 0.62-0.92]; p=0.003). In multivariate analysis, ST-segment deviation (OR 9.9 [95%CI 1.9-51.5]) as well as hematocrit (OR 0.76 per percent [95%CI 0.62-0.92]) remained statistically significant."

Discussion section (on page 12):

"Interestingly, elevation of hsTnT was not related to age, sex, training status, the cardiovascular risk profile or the presence of cardiac arrhythmias during the race, but was related to exercise-induced ST-segment deviation ~~(p<0.0001) and longer marathon finishing time (p=0.040)~~ and inversely correlated with the hematocrit (measured immediately after the race)."

Table 3: Troponin elevation post-marathon in the 107 athletes who finished the marathon race and who had evaluable ECG data.

	Troponin T < 50 ng/L n=89	**Troponin T ≥ 50 ng/L** n=18	**Univariate Analysis** **p-value ***	**Multivariate Analysis** **OR (95%CI)**
[…]				
Hematocrit post-race; %; median [IQR]	44 [42-46]	41 [39-44]	0.003	0.76 (0.62-0.92)

5. *The authors did a cardiac MRI study in a very small subset, but unfortunately this was done too late after the end of the race to detect any transient or reversible abnormalities; nevertheless, these studies performed at 10-42 days later, were negative. It would have been interesting to see echocardiographic findings, much easier to perform, immediately upon finishing the race in the athletes with the abnormal findings. Furthermore, no mention is made about further pursuing assessment of possible ischemic findings in these patients with performance of myocardial scintigraphy and/or CT or regular coronary angiography in these individuals to more definitively exclude coronary artery disease, at least for those with transient ST depression. Were the 9 hypertensive individuals included in this study, which rather should have been excluded from the study, the ones who had the transient ST changes? Did the authors perform regular exercise testing after the race in the individuals with abnormal findings to see whether they were reproducible? Some other assessment examinations that might also have been important in this cohort may include thyroid function studies, and also Holter monitoring and exercise testing before the race that could have provided clues for catecholamine-sensitive arrhythmias, inducible ischemia or hypertensive response, etc.*

We thank the reviewer for this comment. We totally agree that serial echocardiography and exercise capacity testing would have provided additional information, as acknowledged in the limitations (on page 13). However, additional examinations during study conduct were definitively not feasible with regard to more than 100 participating athletes.

As stated in the Methods section (on page 10) “cardiac MRI was offered to all study participants with ST-segment deviation or hsTNT elevation […] and […] additional cardiac work-up was strongly

recommended in all patients with pathological ECG-findings […]". While this additional cardiac work-up was not part of the study, we are unable to comment on the respective findings.

As no athlete (with or without known hypertension) had a hypertensive crisis at baseline, no athlete had to be excluded. Indeed, one out of the nine athletes with known hypertension had a transient ST-segment deviation but no hsTnT elevation during the marathon. In addition, another athlete with known hypertension had a transient hsTnT elevation measured immediately after the race, but no ST-segment deviation during the marathon.

In order to provide a better overview, the main characteristics of 21 athletes with ST-segment deviation and/or hsTnT elevation were included in an additional table in the revised manuscript.

The following information was added to the Results section (on page 8 and 9):

"Characteristics of all athletes with ST-segment deviation are displayed in the *online supplement*. […] Characteristics of all athletes with elevated hsTnT deviation are displayed in the *online supplement*."

ONLINE SUPPLEMENT

High-sensitive troponin T (hsTnT) values returned to normal within up to 58 hours after the marathon race in almost all athletes (95%). Creatine kinase (CK) remains elevated in all 21 athletes (with ST-segment deviation and/or hsTnT elevation).

Table ONLINE SUPPLEMENT: Characteristics of 21 athletes with either ST-segment deviation (>1 mm) and hsTnT elevation (≥50 ng/L) (n=5), ST-segment deviation (n=3) or hsTnT elevation (n=13). Pathological findings are labeled in bold.

Age	Sex	Medication	Cardiovascular risk factors	NSVT[§]	ST-segment deviation	hsTnT[$] ng/L[*]	hsTnT ng/L[**]	CK-MB mg/dL[*]	CK-MB mg/dL[**]	CK mg/dL[*]	CK mg/dL[**]	Cardiac MRI
45	Female	None	No	No	**Yes**	**91**	19	21	32	877	1194	Yes
45	Female	L-Thyroxin	No	No	**Yes**	**71**	12	18	16	243	235	Yes
53	Male	None	No	No	**Yes**	**63**	14	14	35	893	2121	Yes
59	Male	None	No	No	**Yes**	**66**	12	19	54	406	2236	Yes
60	Male	None	Smoker	**Yes**	**Yes**	**70**	12	27	72	2867	3625	Yes
48	Male	None	No	No	**Yes**	32	16	18	44	381	1719	No
55	Male	None	No	No	**Yes**	21	12	21	35	201	489	No
60	Male	Antihypertensive	Hypertension	No	**Yes**	27	13	28	44	767	1979	No
44	Female	None	Smoker	No	No	**78**	12	26	20	306	192	Yes
48	Male	None	No	No	No	**138**	20	59	67	325	2186	Yes
49	Female	None	No	No	No	**63**	12	22	41	205	1357	Yes
50	Male	None	No	No	No	**87**	29	30	73	360	3462	Yes
60	Female	None	No	No	No	**216**	64	33	14	455	169	Yes
39	Male	None	No	No	No	**59**	23	47	87	583	2756	No
40	Female	None	No	No	No	**85**	12	42	33	696	914	No
45	Male	None	No	No	No	**83**	29	175	157	195	605	No
47	Male	None	No	No	No	**52**	12	37	42	309	1983	No
48	Male	None	No	No	No	**64**	12	52	101	405	4604	No
50	Female	Antihypertensive	Hypertension	No	No	**50**	12	29	28	315	485	No
53	Female	None	No	No	No	**62**	12	30	24	339	448	No
54	Male	None	No	No	No	**67**	15	33	23	234	409	No

[§] non-sustained ventricular tachycardia; [$] high-sensitive Troponin T; [*] within 30 minutes post-race; [**] up to 58 hours after the race

6. *Finally, it would have nice to have follow-up data in these patients to see whether any arrhythmic or cardiovascular events ensued during further long-term follow-up, particularly in those with abnormal ECG findings during the race.*

   We have added information on the ECG recordings after the marathon race to the Results section (on page 8) in the revised manuscript.

   "[…] ECG monitoring was prolonged for up to 54 hours (median 28 hours) after the marathon race. ST-segment deviations were not found in any of the eight athletes with ST-segment deviation during the marathon. […]"

The above notwithstanding, the importance of the present study relates to the fact of being able to monitor athletes real-time during their athletic activities, which may enhance our understanding of the effects of strenuous exercise on the cardiovascular system. While exercise can promote health when moderate and regular and performed with gradually increased intensity, it can produce harm when too strenuous and of prolonged duration, specifically incurring cardiac injury and/or dysfunction and consequent electrical instability (Pacing Clin Electrophysiol 2016;39:748-62).

We thank the reviewer for his/her comment.

Reviewer 3:

In this manuscript, Herm et al. report the findings of the "Berlin Beat of Running" study. A cohort of >100 well-trained marathon runners were ECG-monitored and had TnT measured during the Berlin marathon in 2011. They find that one in six athletes develop ECG abnormalities during the race (most commonly nsVT in 9% and ST-segment deviations in 7% of athletes). Interestingly, those with increased TnT after the race were 11-fold more prone to have had ST-segment changes. A cardiac magnetic resonance was performed in 10 athletes (obtained >10 days after the race), in whom no abnormalities were found. The results are interesting.

1. *The authors should provide a clearer definition of their predefined aims in the introduction. As of now, this is only vaguely addressed.*

We thank the reviewer for pointing this out. We have added the following statement to the Methods section (on page 4):

"[…] The study protocol is in accordance with the Helsinki declaration and was approved by the Ethics Committee of the Charité - Universitätsmedizin Berlin (EA4/042/11). The primary hypothesis was: Cardiac arrhythmia and especially AF is frequently found in experienced marathon runners. Therefore, the primary outcome is the number of marathon runners with newly diagnosed cardiac arrhythmias. The main secondary hypotheses were: (1) There are predictable risk factors associated with cardiac arrhythmias in marathon runners; (2) Pathological laboratory findings are in part associated with cardiac arrhythmias; (3) Marathon runners with elevated troponin levels do not have MRI-detected myocardial scars suggestive for myocardial infarction. […]"

2. *ECG filtering plays a critical role in ST-segment interpretation and may lead to a large number of false positives (Tayler D et al. Br Heart J 1985;54:121-128; Abächerli R, Schmid HJ J Electrocardiol. 2009;42:574-9; Buendia-Fuentes et al. ISRN Cardiology 2012:706217). The authors state that the filtering parameters of the monitors allowed to evaluate ST-T wave changes (pg 8, line 15). The filter settings and the specifications should be reported. Also, the authors should report which leads they recorded.*

We thank the reviewer for her/ his remark and have added the following information in the Methods section (on page 5 and 6):

"The cardiologists (AT, AW, WH), who analyzed the ECG data, were blinded for demographic, clinical or laboratory data. The five recorded leads were placed as follows in order to obtain

two independent bipolar channels: left (1) and right (2) on the first intercostal space, right on the sixth intercostal space parasternal (3) and mid-clavicular line (4), left ninth intercostal space mid-clavicular line (5).

[…]

Low-pass-filtering was set at 0.05 Hz in order to detect changes in the ST-T-segment."

3. *Athletes with ST-segment depression at any time during the race are considered abnormal, but this may encompass a large variability (from very short time in some athletes to much longer time-periods in others). Providing results on the duration and the intensity of ST segment depression would substantially improve the manuscript. Moreover, the athletes carried the Holter monitor for >100 hours before and after the marathon. Were ST-segment changes found at any time outside the marathon period?*

   This is an important point raised by the reviewer. Intensity in terms of level in mV was -0.7mV (IQR -0.8 to -0.3, range –0.9 to -0.16). Unfortunately, it was impossible to assess the mean duration of intermittent ST-segment deviation in more detail, as ST-segment changes were present intermittently. Furthermore, in no athlete ST-segment deviation was observed during the ECG monitoring period before and after the marathon race.
   To address the reviewers comment we have added the following information to the Results section (on page 8):
   "Exercise-induced ST-segment deviations occurred in eight (7.5%) study participants during the marathon race (Figure 2), 2 (25%) of those athletes were female. Intensity of ST-segment deviation in terms of ST-level was -0.7mV (IQR -0.8 to -0.3; range -0.9 to -0.16). ECG monitoring was done for up to 54 hours (median 28 hours) after the marathon race. ST-segment deviations were not found in any athlete with ST-segment deviation during the marathon."

4. *An important issue is the significance of ST-segment deviation during a marathon; unfortunately tests aiming at ruling out an ischemic heart disease were not carried out. However, I feel that the specificity and sensitivity for ischemic heart disease of ST-segment deviation in Holter recordings should be more thoroughly discussed to address its significance in athletes. The mechanisms behind Tn elevation after a marathon race have been widely studied in the literature, but up-to-date evidence points to increased permeability of myocytes as a central event (Eijsvogels et al. Physiological Reviews 2016;96:99-125). The authors found a high correlation between ST-segment changes and TnT elevation; do they think that*

ST-segment changes and Tn elevation are both caused by the same factors? Could athletes with both ST-segment deviation and high Tn be at a higher risk of ischemic heart disease?

We are not aware of any study which has systematically assessed the characteristics and etiology of ST-segment deviations in Marathon runners. Our results strengthen the assumption that hsTnT elevation originates from the heart instead of non-cardiac sources such as liver or skeletal muscles, as suggested by Eijsvogels et al. *Physiological Reviews* 2016. However, whether hsTnT elevation is due to an increased permeability of myocytes or cardiac ischemia cannot be clarified by our study. Therefore, we are unable to assess whether athletes with both ST-segment deviation and elevated hsTnT are at higher risk of ischemic heart disease.

We have added the following sentences to the Discussion section (on page 11 and 13):

"Moreover, this is the first study reporting an association of transient ST-segment abnormalities during a marathon with elevated hsTNT levels after finishing the race. However, we cannot be sure that the observed ST-segment deviations are definitively based on silent ischemia."

[…]

"Our results strengthen the assumption that hsTnT elevation originates from the heart and not primarily from non-cardiac sources [8]. However, we are unable to draw final conclusions."

5. *In table 2, and throughout the manuscript, the authors classify athletes with and without an "abnormal ECG". This concept mixes ST-segment depression and nsVT. In my opinion this classification may be misleading as the origin and mechanisms of both events is likely completely different.*

We thank the reviewer for her/his comment. We have pointed out that nsVT and ST-segment deviations were combined for statistical analysis. Indeed, as origin and mechanisms of both ECG alterations may be different, this may have introduced information bias. However, this bias would probably dilute any true association, rendering the reported odds ratios underestimation of the real association.

The following paragraph was added to the limitations section (on page 13):

"Combining nsVT and ST-segment deviations for statistical analysis may have introduced an information bias."

6. *The authors speculate that transient troponin elevation, ST-segment alterations and nsVT are benign (pg 11 line 30) on the grounds of their results. However, the authors would need to provide follow-up data showing similar outcomes in those with and without such events to prove benignity.*

   This is an important point raised by the reviewer. We added the following information in the Methods section, Results section or Discussion section:

   Methods section (on page 5):
   "[…] Follow-up information on past medical history was assessed one year after the marathon."

   Results section (on page 10):
   "[…] Follow-up information was available in all eight athletes with ST-segment deviation and in eight out of ten patients with nsVT during the race. None of the athletes reported a cardiovascular event within one year after the race. […]"

   Discussion section (on page 12):
   "However, athletes with ST-segment deviation or nsVT during the race reported no cardiovascular events within one year afterwards. Thus, these findings are likely to be benign ~~in the absence of~~, but structural heart disease or obstructive coronary artery disease~~, which should~~ was recommended to be ~~excluded~~ ruled out in these athletes […]"

7. *It should be emphasized that a normal cardiac MRI >10 days after does not completely rules out exercise-induced damage. While fibrosis is likely diffuse in athletes (Benito et al. Circulation 2011;123:13-22), this was not addressed with specific cardiac MR techniques in the Herm et al. manuscript. Further, most changes occurring just during a marathon race have regressed 6 to 11 days after the race (La Gerche et al. Eur Heart J 2012;33:998-1006).*

   The reviewer raised a very important point. We agree that the techniques we used: cine steady state free precession (SSFP) and LGE (late gadolinium enhancement) are suboptimal to rule out transient exercise-induced damage. A serial MRI examinations and the use of T2 weighted imaging as well as T1 and T2 mapping techniques would deliver additional information. However please note that functional imaging as performed by SSFP is the standard imaging technique for left and right ventricular function, volumetry and wall motion abnormalities, LGE if present is indicative for acute as well chronic myocardial injury. As mentioned by the reviewer the majority of exercise-induced functional changes will have resolved or regressed after 11 days (as reported by La Gerche et al Eur. Heart J 2012). We assume that the time delays and MRI techniques were optimal for detection of persistent cardiac abnormalities in athletes with abnormal ECG and/or hsTNT elevation. Unfortunately,

we were not able to perform serial cardiac MRI scans in all athletes in addition, because MRI resources were limited.
To better address this issue, we revised the manuscript accordingly (on page 13):

"[…] Major limitations of the observational "Berlin Beat of Running" study, as it focused primary on the feasibility of portable ECG monitoring and detection of ECG changes during the race, are the missing (serial) echocardiography or cardiac stress MRI, limiting the clinical significance of the observed ST-segment changes and elevated hsTnT levels. Furthermore, a normal cardiac MRI within days after the race does not completely rule out (transient) exercise-induced cardiac damage [16]. […]"

8. Minor comments:

Although I assume that a 1 mm was used as a threshold for ST-segment depression, this should be specifically reported in the manuscript.

We thank reviewer and revised the paragraph in the Methods section (on page 6):

"The ST-segment was considered abnormal in the virtually artifact free 2-lead ECG if horizontal or down-sloping ≥ 1 mm occurred over the 60 ms after the J-junction (80 ms if the heart rate was <120 beats/min) [17]."

Herm et al. state that incidence of myocardial infarction after running a marathon is limited to case reports" (pg4 , line 23), but this was addressed in a recent French registry (Gerardin et al. European Heart Journal 2016;37:2531–2541).

We are thankful for this comment and have revised the Introduction section (on page 3) accordingly by mentioning the prospective *RACE* Paris Registry:

"[…] Available data on the incidence of myocardial infarction after running a marathon is limited to case reports and the prospective *RACE* Paris Registry [14], but […]. "

VERSION 2 – REVIEW

REVIEWER	Prof. Martin Burtscher, MD, PhD University of Innsbruck Dept. of Sport Science, Medical Section Austria
REVIEW RETURNED	07-May-2017

GENERAL COMMENTS	I really appreciate the efforts spent by the authors in revising their paper. They responded adequately to most of the points raised. Of course, some weaknesses remain due to the study design as correctly pointed out by the authors. I do not have further comments.

REVIEWER	Antonis S. Manolis, MD Athens University School of Medicine, Athens, Greece
REVIEW RETURNED	22-Apr-2017

GENERAL COMMENTS	Revised manuscript much improved. Thanks for the opportunity to review

REVIEWER	Eduard Guasch Hospital Clinic, Barcelona.
REVIEW RETURNED	02-May-2017

GENERAL COMMENTS	The authors have appropriately addressed most of my concerns and, in my opinion, significantly improve the manuscript. Only some minor comments remain. It should be more clearly stated that, although ST-segment deviation and ventricular arrhythmias are analyzed together, they might be caused by completely different mechanisms and so multivariate analyses could be inaccurate (predictors could be related only to ST-segment deviation, or ventricular arrhythmias). The lack of data on duration or average ST-segment deviation should be considered a limitation. In page 9, line 28: the maximum ST-segment depression mean/median (please report) is -0.7 mV, with range (-0.9 to -0.16). The mean/median is not within the range. Please, correct.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1:

I really appreciate the efforts spent by the authors in revising their paper. They responded adequately to most of the points raised. Of course, some weaknesses remain due to the study design as correctly pointed out by the authors. I do not have further comments.

We thank the reviewer for this comment and his efforts.

Reviewer 2:

Revised manuscript much improved.

We are very thankful for that comment.

Reviewer 3:

The authors have appropriately addressed most of my concerns and, in my opinion, significantly improve the manuscript. Only some minor comments remain.

It should be more clearly stated that, although ST-segment deviation and ventricular arrhythmias are analyzed together, they might be caused by completely different mechanisms and so multivariate analyses could be inaccurate (predictors could be related only to ST-segment deviation, or ventricular arrhythmias).

This is an important issue raised by the reviewer. Therefore, we have revised our manuscript in the Discussion section (on page 12):

"In our prospective study, 10 (9.4%) out of 107 ~~leisure~~ recreational endurance athletes had a non-sustained ventricular arrhythmia during the marathon and one (0.9%) athlete had AF. In addition, ST-segment deviations (Figure 2) were detected in 8 (7.5%) athletes. Advanced age was associated with abnormal ECG findings (Table 2). According to the hematocrit, low hydration – potentially impacting on cardiac preload – was not linked to abnormal ECG findings. However, analyzing athletes with ST-segment deviations, atrial fibrillation or ventricular arrhythmias, the underlying mechanisms of these pathological conditions may differ and the results of the multivariate analysis do not apply to a single condition.

[…]

Combining nsVT and ST-segment deviations for statistical analysis may have introduced an information bias."

The lack of data on duration or average ST-segment deviation should be considered a limitation.

We have added this information to the Limitations section (on page 14):

"Combining nsVT and ST-segment deviations for statistical analysis may have introduced an information bias. Unfortunately, it was impossible to assess the mean duration of ST-segment deviation in more detail, as ST-segment changes were present intermittently. Finally, exercise capacity testing would have allowed more accurate evaluation of training status."

In page 9, line 28: the maximum ST-segment depression mean/median (please report) is -0.7 mV, with range (-0.9 to -0.16). The mean/median is not within the range. Please, correct.

We have double-checked the provided information (on page 9). To our mind, the statement: "Intensity of ST-segment deviation in terms of ST-level was -0.7 mV (IQR -0.8 to -0.3; range -0.9 to -0.16)." is correct as -0.7 mV is within the range of -0.9 to -0.16 mV.